RESEARCH DAY 2012 COMPETITION WINNERS

Twenty-eight research projects were presented orally or by poster by Faculty, Fellows, Residents, Students, and Research Staff. The following competing trainees were named as Award Winners:

(P.I. names are underlined)

Talks

First Place: **John Melville**, M.D., M.S., Child Abuse Fellow (PGY 5)
"The effect of self-blame on trauma symptoms in sexually abused children," with Nancy Kellogg

Second Place: **Judy Chang**, B.S., Graduate Student 3 (PhD Program, Cancer Track)
"Targeting ubiquitin carboxyl-terminal esterase L1 (UCHL1) in neuroblastoma stem-like cells," with Anne Romeo, Aaron Sugalski, Patricia Sanchez-Diaz, Jaclyn Hung

Third Place: **Brian Ely**, M.D., Endocrinology Fellow (PGY 6)
"Effect of Acute Aerobic Exercise on Mammalian Target of Rapamycin (mTOR) Phosphorylation in Human Muscle," with Puntip Tantiwong and Nicolas Musi (Medicine/Diabetes)

Posters

First Place: **Ana Paez**, M.D., Endocrinology Fellow (PGY 5)
"Chronic exenatide infusion increases alpha-beta-delta cell mass in partially pancreatectomized non-human primates," with Subhash Kamath, Francesca Casiraghi, Alberto Davalli, Gregory Avedis Abrahamian, Andrea Ricotti, Raul Bastarrachea, Anthony Commuzzie, Michael Owston, Francesco Andreozzi, Giuseppe Daniele, Paolo Fanti, Ralph DeFronzo, Amalia Gastaldelli, Edward Dick, Glenn Halff, Franco Folli (Medicine/Diabetes)

Second Place: **Emily Moses**, M.D., M.S., Hematology/Oncology Fellow (PGY 5)
"Preclinical studies of salinomycin as a therapeutic agent for glioma cancer stem cells," with Gail Tomlinson and Jaclyn Hung

Third Place: **Maria Rayas**, M.D., Endocrinology Fellow (PGY 5)
"Improving Screening and Care for Cystic Fibrosis-Related Diabetes: A Quality Improvement Initiative," with Donna Beth Willey-Courand and Jane Lynch

Each winner will receive a certificate of award signed by the Department Chair and the Associate Chair for Research, accompanied by a cash prize.

Thanks to all participants for sharing their studies and conclusions, to the judges for volunteering time and effort to the assessment of the presentations, and to all attendees for their support of this annual event!

John Melville, MD, MS,
First Place Oral Presentation

ABSTRACT

The effect of self-blame on trauma symptoms in sexually abused children

John Melville, MD, and Nancy Kellogg, MD

Objective: Sexual abuse is a prevalent and morbid adverse experience in childhood. Prior work suggests that trauma symptoms following sexual abuse are highly variable. In this paper, we report the effect of a child's attribution of blame for the abuse on trauma symptoms following childhood sexual abuse.

Methods: Retrospective review of 501 visits by children age 8-17 presenting subacutely to a community CAC after disclosure of sexual abuse. Abstracted data includes child's attribution of blame for the abuse, parent or guardian's belief in the disclosure, inquiry about trauma symptoms, and the Trauma Symptom Checklist in Children (TSCC-A).

Results: Children who report increasing levels of self-blame for abuse report increased levels of trauma symptoms and higher TSCC-A scores in a dose-response fashion when controlling for age, gender, severity of abuse, familial vs non-familial offenders, and length of abuse. Children whose nonoffending parent or guardian reports not completely believing the disclosure the first time they heard about it are significantly more likely to blame themselves for the abuse.

Conclusion: Self-blame is a significant predictor of trauma symptoms following sexual abuse. Parental belief is an important predictor of self-blame. Child self-blame is a mediator of trauma symptoms following childhood sexual abuse with important clinical, research, and educational implications.

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Ana Paez, MD
First Place Poster Presentation

ABSTRACT

Chronic exenatide infusion increases insulin sensitivity, decreases insulin secretion and stimulates α-β-δ proliferation in partially pancreatectomized non-human primates.

Authors: **Ana M. Paez**, Subhash Kamath, Francesca Casiraghi, Alberto Davalli, Gregory Avedis Abrahamian, Andrea Ricotti, Raul Bastarrachea, Anthony Commuzzie, Michael Owston, Francesco Andreozzi, Giuseppe Daniele, Paolo Fanti, Ralph DeFronzo, Amalia Gastaldelli, Edward Dick, Glenn Halff, Franco Folli

Institutions: San Antonio, TX. Milan, Italy. Pisa, Italy

Baboons represent an interesting non-human primate model of insulin resistance and type 2 diabetes. The study's aims were to assess the effect of continuous EXE infusion after partial pancreatectomy (PP), on in-vivo glucose metabolism, α-β-δ cell mass and islet function in baboons. 4 study groups: 1) EXE (n=12F) & 2) saline (SAL) (n=12F) both underwent PP (the pancreas tail, ~33% of pancreas, was excised); 3) SHAM (no PP) (n=4F); 4) necropsy controls (n=8F & 8M). At baseline, the first 3 groups had a 2-step hyperglycemic clamp (1^{st}: +125mg/dl; 2^{nd}: +225 mg/dl above fasting glucose) + Arginine (0.5g/kg/body weight). Afterwards, a continuous EXE (0.014 ug/kg/h) or normal SAL infusion was started for 13 weeks. After 72 h EXE/SAL washout, clamps were repeated followed by euthanasia and removal of remaining pancreas. Insulin sensitivity (M/I) increased by (↑) 41%, insulin secretion decreased by (↓) 25% and β-cell function=Disposition Index (ISR*M/I) ↑ 50% after EXE. They were unchanged in SAL & SHAM groups. Morphometric analysis was performed with CAST to quantify Islet and immunostained α, β, δ cell volume expressed as a % of total pancreas. PP SAL baboons had 42% ↓ islets in the head-body (6.0 0.6 vs 3.4 0.4, p= 0.01), 43%↓ β-cells (5.5 0.5 vs 3.2 0.2, p=0.003), 74%↓ α- cells (2.4 0.5 vs 0.6 0.2, p=0.003) and 53%↓ δ-cells (0.7 0.1 vs 0.3 0.01, p=0.02) when comparing tail vs head-body. The necropsy control group demonstrated a similar 37% ↓ islets in head-body vs tail (4.9 0.8 vs 3.1 0.2, p=0.03) consistent with a neutral SAL effect. By contrast, in EXE there was only a 5%↓ in islets in the head-body (6.5 0.8 vs 6.2 0.9, p= NS), 17%↓ in β-cells (5.6 0.8 vs 4.7 0.478, p=NS), 21%↓ in α- cells (2.1 0.4 vs 1.6 0.5, p=NS) and 15%↑ in δ-cells (0.7 0.2 vs 0.8 0.01, p=NS) when comparing tail vs head-body. Chronic EXE significantly improves insulin sensitivity, decreases insulin secretion and increases α-β-δ cell mass in non-human primates' pancreas.

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Judy C. Y. Chang
Second Place Oral Presentation

ABSTRACT

Targeting ubiquitin carboxyl-terminal esterase L1 (UCHL1) in neuroblastoma stem-like cells

Judy C.Y. Chang[1], Anne Romeo, Aaron J. Sugalski[1,2], Patricia C. Sanchez-Diaz[1], Jaclyn Y. Hung[1,2]

[1]Greehey CCRI, University of Texas Health Science Center at San Antonio (UTHSCSA), [2] Division of Hematology and Oncology Department of Pediatrics, UTHSCSA

Neuroblastoma (NB) is the most common extracranial solid tumor in childhood cancers. The 5-year survival of patients with advanced disease is <40%, and relapse occurs in over 50%. Evidence suggests that most cancers contain a "stem-like" population that is resistant to current therapeutics and can repopulate the tumor bulk after treatment. This cell population with stem-like characteristics is thought to be an underlying cause for cancer relapse. Therefore, targeting the cancer stem-like cell (CSC) fraction can result in treatment improvements. Previous proteomic work identified that UCHL1 (ubiquitin carboxyl-terminal esterase L1) was differentially expressed in the enriched CSC fraction of human NB cell lines, suggesting a possible link between UCHL1 and CSCs. UCHL1 is part of the ubiquitin proteasome system (UPS), which is responsible for degradation of proteins involved in biological processes such as cell cycle control and the breakdown of transcription factors. Deregulation of UCHL1 expression has been shown to be associated with oncogenic properties; however, the function of UCHL1 in cancer progression is still not fully understood.

We blocked the hydrolase activity of UCHL1 using the small molecule inhibitor LDN-57444. We treated the CSC fraction of NB cell line, SK-N-BE(2), with the inhibitor and observed an impairment of sphere formation (read-out for self-renewal). It has been previously shown that LDN-57444 induces cell death through apoptosis. We explored whether impaired sphere formation was also due to apoptosis. We found through Annexin V staining and measurement of caspase 3/7 activities that the percentage of apoptotic cells was not statistically significant between treated and control CSC cells. We then performed cell cycle studies on inhibitor treated CSCs, which demonstrated an increase in the percentage of cells in the G_1 phase. This suggests that either the inhibitor is causing an arrest at the G_1/S phase or a delay in the cell cycle at the G_1 phase. The cell cycle is an important process in self-renewal, division, and cell differentiation. To further explore the function of UCHL1 in NB CSCs, global gene expression analysis was performed on inhibitor treated CSCs using a PCR microarray with probes specific for neurogenesis and neural stem cells. Results indicated that several cell cycle regulator genes and members of the Wnt/β-catenin/TCF/LEF signaling pathway were deregulated. Among these were *MYC, CCND2, LEF-1, TCF7L2,* and *CDKN2A*. Likely, UCHL1 may be functioning through the Wnt/β-catenin/TCF/LEF pathway to affect *MYC* expression, which would indirectly regulate the cell cycle. *MYC* has also been shown to be a difficult therapeutic target; therefore, UCHL1 will be a more viable target.

We also explored the invasive and tumorigenic potential of SK-N-BE(2) CSCs. An invasion assay performed on inhibitor treated CSCs showed that there was not a significant difference in the number of invasive cells between drug and control. Similarly, a soft agar assay was performed on inhibitor CSCs, but no significant effect was seen in the CSCs. This could suggest that a more resistant population is being selected. In addition to UCHL1, a secondary therapeutic target may be necessary to affect the CSC population.

Targeting the UPS, which functions in cell development and survival, has been shown to be successful in some cancers. These observations suggest that UCHL1 functions in the maintenance of CSCs, and targeting UCHL1 in combination with secondary target could lead to better design of therapeutic intervention that improves quality of life and long-term survival of NB patients. (Supported in part by a Greehey Graduate Fellowship in Children's Health (Greehey Family) to CJC).

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Emily Moses, MD
Second Place Poster Presentation

ABSTRACT

Preclinical studies of salinomycin as a therapeutic agent for glioma cancer stem cells

Emily S. Moses, Gail E. Tomlinson and Jaclyn Y Hung

Background: Brain tumors are the most common childhood solid tumors. There are many types of brain tumors; the most common are gliomas. Gliomas are grouped as astrocytomas, oligodendrogliomas or ependymomas. This study is focused on astrocytomas. In the low-grade astrocytomas complete surgical resection has achieved over 90% five-year survival rate. But in cases that are not fully resected, cytotoxic adjuvant therapies is needed to prevent relapse. The aggressive anaplastic astrocytoma high-grade 3 and the fast growing glioblastoma multiforme high-grade 4 are often refractory to standard therapies, with less than 4% of these patients alive after 5 years. Aggressive surgical procedures and adjuvant therapies, especially in young children, can have devastating neurocognitive sequelae, indicating a need for improved and novel therapies. Salinomycin, an antibiotic potassium ionophore, has recently been shown to be highly effective against breast cancer stem cells and other malignancies; however, its potential use has not been studied in childhood astrocytomas.
Objective: To determine whether salinomycin will be effective in eradicating growth of resistant brain cancer cells in a pre-clinical setting.
Design/Methods: Two pediatric glioma cell lines were utilized. Res186 was kindly provided by Dr. John Silber (University of Washington). Dr. Daphne Haas-Kogan (UCSF) provided SF188. The cells were incubated in the presence of different concentrations of salinomycin and the IC_{50} was determined. The ability of the cells to form colonies in soft agar, apoptosis, and cell cycle distribution in the presence of salinomycin were measured. To investigate the effect on the stem-like cell component, cells were incubated in the presence of salinomycin and the ability to form spheres or the percentage of CD133 positive cells was determined. To evaluate statistical significance we performed unpaired Student's *t* test and *P* values <0.05 were used as cut off for significance.
Results: Our results indicated that the cells were sensitive to salinomycin. The soft-agar anchorage independent growth assay showed that salinomycin impaired *in vitro* proliferation for both cell lines. In SF188 cells treated with salinomycin, the percentage of CD133 positive cells, a marker for "stemness", was decreased and the number of cells in S-phase was decreased. Additional studies on Res186 and on apoptosis for both cell lines are ongoing.
Conclusion: The data obtained from this study may provide the basis for novel and potentially highly effective treatment of these childhood malignancies.

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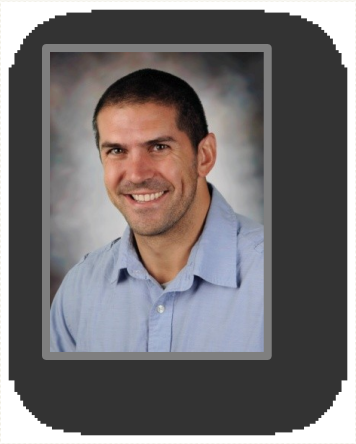

Third Place Oral Presentation

ABSTRACT

Brian Ely, Puntip Tantiwong, Nicolas Musi
UTHSCSA, Texas Diabetes Institute

Effect of Acute Aerobic Exercise on Mammalian Target of Rapamycin (mTOR) Phosphorylation in Human Muscle

Mammalian target of rapamycin (mTOR) is a nutrient-sensitive Ser/Thr kinase that controls protein and glucose metabolism. In skeletal muscle, the mTOR signaling pathway is stimulated by resistance exercise and this is thought to promote an increase in muscle mass. However, the effect that aerobic exercise has on mTOR, particularly in man, is less well known. In this study we examined the effect that acute exercise has on mTOR phosphorylation in human subjects. Six subjects (age= 42±4 years, BMI=30±0.7 kg/m2, fasting plasma glucose=84±3 mg/dl, plasma insulin=18±10 mU/L) exercised on a bicycle for 40 min at 70% VO2max and vastus lateralis muscle biopsies were obtained 30 min before exercise, immediately after 40 min of exercise, and after 210 min of rest. mTOR phosphorylation (Ser2448) and protein content were measured by Western analysis. We found that exercise increased mTOR phosphorylation (p-mTOR/mTOR) by 1.9-fold (prexercise=0.79±0.14 AU; 40 min time point=1.54±0.23 AU; P<0.05). After 210 min of rest, mTOR phosphorylation had returned to basal levels (0.82±0.14 AU). In summary, aerobic exercise rapidly and transiently phosphorylates mTOR in human subjects. Activation of the mTOR signaling pathway could be a mechanism by which aerobic exercise regulates muscle protein and glucose metabolism.

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Maria Rayas, MD

Third Place Poster Presentation

ABSTRACT

Improving Screening and Care for Cystic Fibrosis-Related Diabetes: A Quality Improvement Initiative

Maria Rayas, MD, Donna Beth Willey-Courand, MD**,** and Jane Lynch, MD
No funding

Background: Cystic fibrosis-related diabetes (CFRD) is a leading CF co-morbidity associated with accelerated nutritional and pulmonary decline, microvascular complications, and increased mortality. CFRD screening and aggressive management is imperative. An annual 2-hr oral glucose tolerance test (OGTT) is recommended for CFRD screening in CF patients starting at 10 years of age, and those patients with 1st or 2nd degree relatives with Type 2 DM may benefit from earlier screening. Guidelines now acknowledge an indeterminate (INDET) OGTT category in which the 1hr glucose is ≥ 200mg/dl and a recent study demonstrated that INDET results are associated with a decline in lung function. Previous studies suggest that the OGTT underestimates hyperglycemia and continuous glucose monitoring (CGM) provides an alternate method for the early detection of hyperglycemia in CF patients. CFRD screening can be a challenging component of care to consistently order, track, and interpret. Beginning in August 2010, the CHRISTUS Santa Rosa Children's Hospital CF Center devised a quality improvement algorithm to improve screening of CFRD which has spearheaded by a pediatric endocrine fellow who joined the CF management team. The algorithm included the use of CGM in select patients with abnormal (IFG/IGT or INDET) OGTT results to determine if early treatment with insulin could be beneficial.

Specific Aim: To implement a CFRD screening algorithm sheet that would result in screening 100% of CF patients aged 8-17 years for CFRD at our CF center between March 8, 2011- September 8, 2011

Methods: The CFRD Screening Algorithm was placed in the chart of qualifying patients during the 6 month period. The endocrine fellow tracked the results. The CF Center director and team implemented a plan for management of abnormal results and all CFRD patients received ongoing care by the endocrine fellow at the CF clinic.

Summary of Results: The algorithm was placed in 98.3% of CF patients aged 8-17 years in our CF center. Of those patients, 31% already had the diagnosis of CFRD and no further workup was performed. Ninety-seven percent were appropriately screened with an OGTT. Twenty-percent of patients had NGT, 15% had IGT, 10% had INDET, 5% had CFRD, and 14% were pending results. Seventy-one percent of 8-9 year olds had NGT. One 8 year old with INDET results had a 1st degree relative with Type 2 DM. One of 3 patients with INDET had an abnormal CGMS and was started on insulin.

Conclusions: The CFRD Screening Algorithm helped develop a consistent process to screen for CRFD and track the results obtained from the OGTT and CGMS. The majority of CF patients between 8-9 years of age had NGT results on OGTT an my not benefit from early screening unless they have a 1st or 2nd degree relative with Type 2 DM. CF patients with INDET may benefit from CGMS placement. Prospective studies on the benefits of initiating insulin in select patients with INDET results and abnormal CGMS need to be performed. Screening for and management of CFRD by an endocrinologist trained in CF and present in the CF clinic at the time of CF appointments may be beneficial and very convenient for CF families.

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